Multiparametric MRI evaluation of kainic acid-induced neuronal activation in rat hippocampus

Yi-Hua Hsu,1,* Wang-Tso Lee3,* and Chen Chang1,2

1Institute of Biomedical Sciences, Academia Sinica, Taipei, 115, 2Institute of Pharmacology, National Yang-Ming University, Taipei, 112 and 3Department of Pediatrics, National Taiwan University Hospital, Taipei, 100, Taiwan

*These authors contributed equally to this work.

Correspondence to: Dr Chen Chang, Institute of Biomedical Sciences, Academia Sinica, Taipei, 115, Taiwan, R.O.C.
E-mail: bmcchen@ibms.sinica.edu.tw

We investigated the pathogenic mechanisms of hippocampal structural changes and neuronal activation in a kainic acid (KA)-treated rat model using non-invasive high-resolution diffusion-weighted imaging, T2-weighted imaging, and manganese-enhanced magnetic resonance imaging (MEMRI). We found that high-resolution MRI can reveal KA-induced subtle lesions in hippocampus. The signal changes were first observed in the CA3 area and then the CA1 area, and were revealed to be focal edema and neuronal death in histopathological studies. MR signal intensity was higher in CA1 area than in CA3 area at 168 h post KA treatment due to the increase of proliferating astrocytes as shown by histopathological studies. MEMRI studies revealed signal hyperintensity in the CA3 pyramidal cell layer after KA treatment, and the MEMRI signal can be attenuated by diltiazem, an L-type calcium channel blocker. Histopathological study revealed attenuation of focal edema and neuronal swelling following diltiazem treatment. It indicated that KA-induced neuronal activation mainly developed in CA3, and calcium channels may play important roles in pathogenesis of KA-induced hippocampal lesions. We conclude that high-resolution MRI is able to identify KA-induced hippocampal damage, and that MEMRI can be used to investigate the role of calcium channels in the pathogenic mechanisms of neurological conditions.

Keywords: neuronal activation; high resolution MRI; manganese-enhanced MRI; kainic acid; c-Fos

Abbreviations: BBB = blood-brain barrier; DWI = diffusion-weighted imaging; KA = kainic acid; MEMRI = manganese-enhanced MRI; SI = signal intensity; T1WI = T1-weighted imaging; T2WI = T2-weighted imaging; TLE = temporal lobe epilepsy; VGCC = voltage-gated Ca2+ channel


Introduction

Temporal lobe epilepsy (TLE) is the most common epilepsy in adults (Sloviter, 2005), and is also very common in children. It is characterized by recurrent seizures and subsequently neuronal death in the hippocampus (Buckmaster, 2004). To understand the pathogenic mechanisms of seizure-induced brain damage is important in the management of epilepsy (Ben-Ari and Cossart, 2000; Andrade et al., 2006). Several animal models of TLE have been designed (Zucker et al., 1983; Ben-Ari, 1985; Sloviter, 2005). Of these, kainic acid (KA)-induced model is one of the most commonly used TLE models (Ben-Ari, 1985; Sloviter, 2005). The seizures induced by KA in rats are usually prolonged and generalized, and are different from those in human TLE (Sloviter, 2005). The KA-induced hippocampal lesions are also somehow different from those in human TLE (Sloviter, 2005). However, electrophysiological studies in KA animal model had shown the rising of epileptiform activity from the hippocampal CA3 region within 1 h of KA treatment (Lothman et al., 1981; Ben-Ari, 1985). Histological studies also demonstrated that KA-induced seizures were accompanied by blood–brain barrier (BBB) disruption (Zucker et al., 1983; Ruth, 1984), and neuronal death in the hippocampus (Sperk, 1994). Although KA may produce more extensive brain damage than hippocampus (Sloviter, 2005), KA-induced hipocampal cell loss followed by abnormal synaptic reorganization has been postulated to be the source of chronic spontaneous seizures (Dudek et al., 2002). Therefore, KA-induced hippocampal injury remains one of the most commonly used models in investigating the pathogenic mechanisms of TLE in human.

In vivo observation of the evolution of KA-induced hippocampal lesions is helpful for our understanding of epileptogenesis and developing early diagnostic tools and therapies for TLE. Non-invasive magnetic resonance
imaging (MRI) technique allows longitudinal studies of structural and functional changes in the same brain (Le Bihan, 2000; Lee and Chang, 2004). Diffusion-weighted imaging (DWI) and T2-weighted imaging (T2WI) have been used to assess the histopathological changes in rat model of KA-induced TLE. However, significant MR changes were not observed until 24 h after KA-induced seizures (Wang et al., 1996). The discrepancy between histological and MRI results may be due to insufficient MR resolution to detect seizure-induced hippocampal lesions, which may only develop in specific region of the hippocampus (Righini et al., 1994; Nakasu et al., 1995; Wang et al., 1996).

Recently, manganese (Mn2+) -enhanced MRI (MEMRI), a functional imaging tool, has been used to detect brain activation in response to drug stimulation (Lin and Koretsky, 1997; Aoki et al., 2004a; Yu et al., 2005; Kuo et al., 2006; Lu et al., 2007). Mn2+ acts as an MRI contrast agent by shortening T1, and can enter neurons as an analogue of calcium (Ca2+) through voltage-gated Ca2+ channels (VGCC) during neuronal activation. Thus, activated neurons will contain higher Mn2+ concentrations, and reveal increased signal intensity (SI) in MEMRI. Because VGCCs are of great importance in pathogenesis of various forms of epilepsies and may modulate seizure initiation, propagation, termination, and kindling (Khosravani and Zamponi, 2006), MEMRI may be used to evaluate the role of VGCCs in seizure initiation.

To investigate the pathogenic mechanism of KA-induced hippocampal injury, in the present study we used high-resolution DWI and T2WI to evaluate the development of KA-induced subtle hippocampal lesions. MEMRI was also used to identify KA-induced neuronal activation in the hippocampus, and evaluate the role of VGCCs in KA neurotoxicity and seizure initiation.

Materials and methods

Animal preparation

Adult male Sprague-Dawley rats (250–330 g; National Laboratory Animal Center, Taiwan) were used in the present study. Animals were anaesthetized using chloral hydrate (450 mg/kg, Sigma, MO, USA), intubated and polyethylene catheters (PE-50, Becton Dickinson, CA, USA) were then inserted into the femoral vein for drug administration. For MRI experiments, rats were maintained under anaesthesia using 1.4% isoflurane in a 1 l/min oxygen flow. To minimize the impact of KA-induced body tremor on image quality, one incisor bar and two ear bars were used to firmly fix the rat head in the custom-designed head holder. Tape was also used to truss rat head and holder up for immobilization. Rectal temperature was maintained at ~37°C using a warm water blanket and by injecting warm air through the bore of the magnet. pCO2 and pO2 in the animals were continuously monitored using Capnomac Ultima-I (Datex-Ohmeda) to keep at constant level in each experiment.

To study the development of KA-induced hippocampal lesions, MR scanning was sequentially performed on the same rat (n = 6) before and at 0–1, 1–2, 2–3, 24, 48, 72, 120 and 168 h after a bolus intravenous (i.v.) injection of KA (12 mg/ml/kg, Sigma, MO, USA) (Fig. 1). The same MRI experimental protocol was also applied on the control rats without KA treatment (n = 6). To assess KA-induced BBB disruption, five rats were twice administered a bolus i.v. injection of Gd-DTPA (0.2 mmol/kg, Magnevist, Germany) at 1 h 45 min and 2 h 45 min after KA administration (Fig. 1). Another five rats without KA administration were also injected with Gd-DTPA for comparison. To investigate the neuronal activation following KA administration, MEMRI was done by i.v. administering Mn2+ (0.5 mmol/kg, Sigma, MO, USA) into rats (n = 5) for 3 h immediately post-KA treatment (Fig. 1). Five rats were infused with Mn2+ (0.5 mmol/kg) for 3 h without KA treatment as the controls. Diltiazem, a VGCC blocker (Sigma), was also used to clarify whether the change of SI in MEMRI was related to neuronal activation (n = 5) (Lu et al., 2007). The rats were sacrificed after scanning for histological examinations. All procedures were approved by the Institute of Animal Care and Utilization Committee in Academia Sinica, Taiwan.

MRI acquisition and data processing

All MRI experiments were performed at 7 Tesla PharmaScan 70/16 with an active shielding gradient at 300 mT/m in 80 μs, with rats in a prone position. A 38 mm i.d. volume coil was used for signal excitation and receiving. A series of DWI, T2WI and T1-weighted imaging (T1WI) images were repeatedly acquired before and after KA injection (scheme illustrated in Fig. 1). As ventral hippocampus is highly vulnerable to KA (Schwob et al., 1980) and essential for KA-induced behavioural changes (Grimes et al., 1990), axial images containing ventral hippocampus were obtained at a distance of 5.2 mm posterior to the Bregma (Paxinos and Watson, 1998) with a field of view (FOV) of 2.56 cm, a slice thickness of 1 mm and a matrix size of 256 × 128. The image slice position was determined by comparing the images with rat brain atlas (Paxinos and Watson, 1998). After image acquisition, images were zero-filled to 256 × 256, resulting in an in-plane resolution of 100 μm × 100 μm. For DWI, a Stejskal-Tanner spin-echo sequence was used [repetition time (TR) = 1500 ms, echo time (TE) = 34 ms, diffusion gradient duration = 3.5 ms, diffusion gradient separation = 15 ms, b value = 1100 mm2/s along the X direction, number of excitations (NEX) = 8]. For T2WI, a fast spin-echo sequence was used (TR = 4000 ms, echo train length = 8, effective TE = 70 ms, NEX = 16). For T1WI, a spin-echo sequence was used (TR = 600 ms, TE = 10.3 ms, NEX = 12).
All data analyses were performed using MRVision equipment (MRVision Co., CA, USA). To study KA-induced MR signal changes in hippocampal layers, regions of interests (ROIs) were traced carefully on DWI scans based on the stereotaxic atlas (Paxinos and Watson, 1998), and the findings on immunostaining of VGCC (Fig. 2), and then automatically transferred to T2WI. Because of the possible morphological alterations in vivo after KA treatment and during histochemical procedures, we analysed the data carefully as suggested previously (Fabene et al., 2003), to correlate the MRI SI changes with histological alterations as described subsequently. The sequential changes of hippocampal subregions in MR studies were analysed in the same animal in different time points. To eliminate measurement errors, two well-trained imaging analysts, who were blind to the state of the rats under investigation, were responsible for ROI depicting. The secondary visual cortex, a brain region resistant to KA neurotoxicity (Schwob et al., 1980), in the same image was chosen as the control ROI. In order to eliminate the individual variations in MR SI that arise during data collection, the MR SI is presented as SI ratio, which was calculated by dividing the SI of each hippocampal layer with the SI of the secondary visual cortex and multiplying by 100 (Saleem et al., 2002). Amira (Template Graphics Software, San Diego, CA, USA) was used to produce the color map in order to emphasize the Mn2+-induced hyperintensity in MEMRI studies.

Statistics

Statistical analyses were performed using StatView (SAS Institute Inc., NC, USA). All data are presented as mean ± SD. Repeated measure ANOVAs followed by Fisher’s post-hoc tests were applied to compare the MR signal changes in the hippocampus before and after KA treatment at different time points. Two-way ANOVA followed by Fisher’s post-hoc tests were used to analyse the effects of treatments on MR signal changes over the observed time points. P < 0.05 was considered statistically significant.

Histochemical studies

For correlation of KA-induced MR findings and histopathological changes, 25 rats were used for histology study. After the MR experiments, the rats were sacrificed at 1–2 h (n = 5), 2–3 h (n = 5), 48 h (n = 5) and 168 h (n = 5) after KA treatment for haematoxylin and eosin (H&E) staining and immunostainings of glial fibrillary acidic protein antibody (GFAP) and c-Fos. Five rats without KA treatment were used as control. Another five rats were treated with KA under consciousness and sacrificed at 1–2 h for c-Fos study. All rats were transcardially perfused with 4% paraformaldehyde in phosphate buffered saline (PBS, pH = 7.4). Brains were then removed, and placed in the same fixative overnight. The 20 μm brain sections at positions matching the MR images were then done. Immunostaining with anti-L-type VGCC antibody (1:200; ACC-003, Alomone, Jerusalem, Israel) was applied to demonstrate the hippocampal layers in the rat brain sections. H&E staining was performed to identify KA-induced histopathogenesis. Immunostaining with anti-c-Fos antibody (1:200; PC38, CALBIOCHEM, CA, USA) and anti-GFAP (1:200; Z0334, Dako, CA, USA) was performed to assess neuronal activation (Popovici et al., 1990) and reactive gliosis (Benkovic et al., 2006), respectively. Briefly, after washing with PBS containing 0.3% Triton X-100, free-floating sections were treated with 0.2% H2O2 in PBS to inhibit endogenous peroxidase. Non-specific binding was blocked by incubating sections in 3% normal goat serum plus 2% bovine serum albumin in PBS containing 0.3% Triton X-100 for 1 h.
Sections were then incubated with the primary antibody (1:100) overnight at room temperature, then with a biotinylated goat anti-rabbit antibody (1:1000; Jackson Immuno Research, West Grove, PA, USA) for 1 h. For visualization, sections were then incubated with an avidin-biotin-horseradish peroxidase complex (Pierce, Rockford, IL, USA) for 1 h, and were examined using light microscopy.

**Results**

**Behavioural changes**

KA-induced behavioural changes under anaesthesia were monitored after i.v. administration of 12 mg/kg KA. A well-trained technician stood beside the magnet to observe the KA-induced behavioural changes of the rats without knowing the drug treatment of the rats (n=6 in KA group; n=6 in control group). The KA-induced behavioural changes in both freely moving and anesthetized rats were very consistent in our study. The breathing rate of the rats was around 40–50 breaths/min under anaesthesia, and gradually increased to 50–90 breaths/min within 20 min after KA administration. Between 20–70 min post KA injection, tail shaking and intense hind limb convulsions were first observed as those in freely moving rats. From 70 min post KA injection, rats showed continuous tail/hind limb tremors with a breathing rate of 100–150 breaths/min. In rats treated with diltiazem and KA (n=5), the breathing rate was only mildly increased, and never exceeded 80 breaths/min. The tail shaking and hind limb convulsions were also significantly attenuated. After scanning, the KA-treated rats appeared exhausted while Diltiazem-treated rats appeared more active. These behavioural observations are consistent with previous reports (Ben-Ari, 1985; Sperk, 1994).

**Laminar organization in the intact hippocampus identified using high resolution DWI**

High-resolution DWI and T2WI were used to investigate temporal changes in the hippocampus. Six distinct hippocampal layers, including the CA3 stratum oriens (subregion 1), CA3 pyramidal cell layer (subregion 2), CA3 stratum radiatum (subregion 3), hippocampal fissure (subregion 4), CA1 stratum radiatum (subregion 5) and the CA1 pyramidal cell layer (subregion 6) were identifiable using DWI (Fig. 2A), but not T2WI (Fig. 2B) (n=6). Magnified control hippocampus DWI clearly delineated subregions 1–6, and showed that subregions 1, 3 and 5 appeared to be more hyperintense than subregions 2, 4 and 6 (Fig. 2C). To correlate the hippocampal layers identified by DWI, immunostaining of VGCC was done after MR experiments for both control and experimental rats. Immunostaining of VGCC can demonstrate the hippocampal ultrastructure from subregions 1 to 6 like those in DWI (Fig. 2D) (n=5).

**KA-induced hippocampal lesions revealed by high resolution DWI, T2WI and contrast-enhanced MRI with Gd-DTPA**

A series of high resolution DWI and T2WI studies were repeatedly performed to identify the temporal and spatial characteristics of KA-induced lesion development in hippocampus (Fig. 3) (n=6). As early as 1–2 h post KA injection, both T2WI and DWI revealed increased SI in the hippocampal CA3 region. By contrast, SI in the CA1 region was not increased until 48 h after KA treatment. In control rats (n=6), there was no significant SI change in hippocampal subregions under anesthesia.

Quantitative analyses of KA-induced MR signal changes in each hippocampal layer were shown in Fig. 4. In CA3 neuronal basal dendrites (subregion 1, Fig. 4A) and cell bodies (subregion 2, Fig. 4B), both T2WI and DWI SI ratios rapidly increased at as early as 1–2 h, peaked at 24–48 h, and pseudo-normalized at 168 h. The first significant increase in SI ratios in the apical dendrites (subregion 3, Fig. 4C) occurred at 2–3 h, peaked at 24–48 h, and pseudo-normalized at 120 h. Furthermore, SI ratios of CA3 pyramidal cell bodies (subregion 2) on DWI at 2–3 h, 24 h and 48 h were significantly different from those of CA3 neuronal dendrites (subregions 1 and 3) (P<0.05), indicating that different layers in CA3 had different changes after KA treatment. SI ratio changes in the CA1 region also differed from those of the CA3 region. The first significant increase in SI ratios in the CA1 apical dendrites (subregion 5, Fig. 4D) and cell bodies (subregion 6, Fig. 4E) occurred at 48 h and 72 h, respectively. Moreover, the SI ratios in the CA1 subregion 6 did not return to normal levels as those in CA3, and remained higher than those in CA3 at 120 h and 168 h (P<0.01 and P<0.05, respectively). It suggested that pathogenic mechanisms of KA-induced CA1 damage may be different from those of CA3.

To determine the relationship between SI changes in the CA1 and CA3 regions and BBB disruption, contrast-enhanced MRI with Gd-DTPA was also used to assess BBB permeability in the hippocampus after KA treatment (n=5). Signal enhancement by Gd-DTPA was observed at 2–3 h in the CA3 pyramidal cell layer (subregion 2, 192±34% of the control, P<0.05) (Fig. 5), indicating that different layers in CA3 had different changes after KA treatment. SI ratio changes in the CA1 region also differed from those of the CA3 region. Gd-DTPA administration did not alter the pattern of KA-induced MR signal changes observed using DWI (Figs. 3 and 5).

**Histopathological studies**

To clarify the significance of MR changes, brains were removed at different time points following KA administration and H&E staining was done (Fig. 6) (n=5 for each time point). At 1–3 h post KA injection, gradual swelling of neuronal dendrites in CA3 subregions 1 and 3 (Fig. 6A) was observed, and progressive shrinking of neuronal cell bodies and formation of peri-neuronal vacuoles were detected.
Fig. 3  Time-dependent changes of the KA-induced hippocampal lesions in vivo. A series of high resolution DWI and T2WI images were obtained at a distance of 5.2 mm posterior to the Bregma from the same rat at different times (n = 6). Arrows indicate lesions in the hippocampal layers from subregions 1 to 6.

Fig. 4  Quantitative analyses of temporal MR signal changes in hippocampal subregions after KA administration. The curves illustrate different signal patterns in DWI and T2WI for the different hippocampal subregions after KA administration (n = 6). (A) CA3 stratum oriens (subregion 1). (B) CA3 pyramidal cell layer (subregion 2). (C) CA3 stratum radiatum (subregion 3). (D) CA1 stratum radiatum (subregion 5). (E) CA1 pyramidal cell layer (subregion 6). Data are presented as an SI ratio (%), which was calculated by dividing the SI for each hippocampal layer by the SI for the control cortex and multiplying by 100. Open circles represent DWI SI ratios, and closed circles represent T2WI SI ratios. Asterisk indicates the significant differences between the SI ratios before KA and those after KA at indicated time points, at a level of P < 0.05.
in the CA3 subregion 2 (Fig. 6B), consistent with the increased SI shown in DWI and T2WI (Figs. 3 and 4). Significant neuronal death in the CA3 region was observed at 48 h post KA injection (Fig. 6A), resulting in the vacuolation of CA3 region along with severe edema formation as reflected by the peaks of DWI and T2WI SI ratios. In contrast to the CA3 region, the condensation of CA1 neuronal cell bodies (subregion 6) and the vacuolation in CA1 neuronal dendrites (subregion 5) did not occur until 48 h after KA treatment (Fig. 6C), consistent with the later increase in SI shown in DWI and T2WI of the CA1 area. The degenerated CA3 and CA1 tissues were gradually replaced by proliferating glial cells after 120 h (Figs 6B and C), especially in the CA1 area, leading to the disappearance of the focal edema and peri-neuronal vacuoles as reflected by the pseudo-normalization of DWI and T2WI SI ratios at late stages. Proliferating glial cells in the CA1 area may be responsible for the higher MR SI in this area at 120 h and 168 h. These histopathological findings are consistent with previous investigations (Schwob et al., 1980; Tokuhara et al., 2005), which showed a greater number proliferating glial cells in the CA1 area at 7–21 days post KA treatment. To further investigate the proliferating glial cells in CA1 following KA injection, GFAP staining was performed, and it displayed that GFAP-expressing astrocytes were hypertrophied and increased in the CA1 area at 168 h (Fig. 7) (n = 5 for each time point), supporting the KA-induced reactive gliosis at late stage (Fig. 6).

**KA-induced neuronal activation in the hippocampus**

To investigate the KA-induced neuronal activation in hippocampus, and correlate the SI changes in DWI with neuronal activation, MEMRI was performed on both normal and KA-treated rats. Mn\(^{2+}\) infusion did not...
attenuate KA-induced convulsions, and the behavioural changes in rats treated with KA alone or both KA and Mn²⁺ were also not different. As shown in Fig. 8 and Table 1, Mn²⁺ infusion in control rats \( (n=5) \) only revealed signal enhancement in CA1 pyramidal cell layer and alveus, but not in other hippocampal subregions (Fig. 8A). On the contrary, Mn²⁺-induced hyperintensity was also observed in the CA3 pyramidal cell layer in KA-treated rats (Fig. 8B) \( (n=5) \), and became more intense at 2–3 h post KA injection (Table 1). However, there was no significant difference of MEMRI signal in CA1 for both KA-treated and control rats (Table 1).

To further clarify the mechanisms of MEMRI signals of CA1 and CA3 in KA-treated rats, diltiazem, an L-type VGCC blocker, was co-injected (2.5 mg/kg) with KA, and then co-infused (2.5 mg/kg) with Mn²⁺ for 3 h after KA application (Lu et al., 2007) \( (n=5) \). Diltiazem injection did not change the pattern of MEMRI signal in CA1 for both KA-treated and control rats (Table 1). However, diltiazem significantly attenuated the KA-induced MEMRI signal in CA3 subregion 2, and also decreased KA-induced DWI signals in CA3 (Table 1 and Fig. 8C). Histopathological study revealed that diltiazem treatment significantly reduced focal edema and neuronal swelling in CA3 region (Fig. 9) \( (n=5) \), compatible with the findings in DWI and MEMRI. It indicated that early MEMRI signal in CA3 region may be related to neuronal activation via VGCCs while MEMRI signal in CA1 region was not related to VGCCs.

To further elucidate the region of neuronal activation, and clarify the effect of anesthesia, c-Fos staining in KA-treated and control rats with and without isoflurane anesthesia was also performed \( (n=5 \) for each condition).

As shown in Fig. 10, 1–2 h post-KA injection in waking rats, the expressions of c-Fos were increased in dentate gyrus (DG), CA1 and CA3, consistent with previous report (Popovici et al., 1990). However, in isoflurane-anesthetized rats, c-Fos immunoreactive cells were only significantly increased in the CA3 area \((15.2 ± 7.4 \text{ cells/m}^2)\) versus

---

Table I Mn²⁺-induced signal enhancement in hippocampal subregions under different conditions

<table>
<thead>
<tr>
<th>Subregions</th>
<th>Mn²⁺ only</th>
<th>Mn²⁺ + KA</th>
<th>Mn²⁺ + KA + Dil.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subregion 2</td>
<td>Before</td>
<td>105 ± 3</td>
<td>101 ± 2</td>
</tr>
<tr>
<td>Post 1–2 h</td>
<td>103 ± 7</td>
<td>142 ± 22†</td>
<td>101 ± 9##</td>
</tr>
<tr>
<td>Post 2–3 h</td>
<td>106 ± 6</td>
<td>176 ± 35†</td>
<td>132 ± 22†#</td>
</tr>
<tr>
<td>Subregions 6 and 7</td>
<td>Before</td>
<td>98 ± 2</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>Post 1–2 h</td>
<td>129 ± 14*</td>
<td>133 ± 21*</td>
<td>122 ± 5*</td>
</tr>
<tr>
<td>Post 2–3 h</td>
<td>155 ± 15*</td>
<td>144 ± 28*</td>
<td>148 ± 11*</td>
</tr>
</tbody>
</table>

Values were SI ratios expressed as mean ± SD \( (n=5 \) for each group). 
†\( P<0.05 \), compared with those before KA injection. 
‡\( P<0.05 \), compared with Mn²⁺ only. 
##\( P<0.05 \), ##\#\( P<0.01 \), compared with (Mn²⁺ + KA).
Discussion

The present study demonstrates for the first time that high resolution MRI can reveal not only hippocampal ultrastructures, but also KA-induced hippocampal lesions over time. The temporal changes in MR signals correlated well with histopathological changes in hippocampal layers at the cellular level, and may reflect early signs of hippocampal sclerosis. Using MEMRI and DWI, we also showed that VGCCs may play important roles in KA-induced neuronal activation and neurotoxicity in CA3.

Although KA-induced brain damage is not exactly the same as those in human TLE (Sloviter, 2005; Andrade et al., 2006), KA-treated rats remains one of the major animal models in investigating the pathogenic mechanisms of seizure-induced brain injury. As a non-invasive tool, MR techniques have been widely used in evaluating neuronal damage (Lee et al., 2000; Lee and Chang, 2004), and can long-term investigate the pathological changes in the same brain, which is more informative and not possible using histopathological techniques. Previous in vivo MRI investigations were unable to identify acute hippocampal lesions after KA treatment, probably due to insufficient image resolution (180 × 180 mm²) (Righini et al., 1994; Nakasu et al., 1995; Wang et al., 1996). The present study shows for the first time that in vivo high-resolution DWI at 100 × 100 µm² (interpolated from a matrix size of 256 × 128 to 256 × 256 by zero-filling) was sufficient to identify distinct hippocampal ultrastructure.

The ability to detect hippocampus laminar structure is helpful to our understanding of pathogenic mechanisms of the KA-induced lesions in hippocampus. The early increase in SI ratios at 1–2 h post KA injection in the CA3 pyramidal cell layer (subregion 2) may reflect KA excitotoxicity on the cells. KA selectively activates kainate receptors (KAR), which are preferentially expressed on the proximal dendrites (mossy fibre synaptic region) of CA3 pyramidal neurons (Jaskolski et al., 2005). The activation of KARs by KA may activate VGCCs and generates epileptic activity in CA3 pyramidal cells, leading to acute KA neurotoxicity in the CA3 subregion 2 (Coyle, 1983; Albowitz et al., 1997; Castillo et al., 1997). The activation of KARs also allows Na⁺ influx and K⁺ efflux (Arundine and Tymianski, 2003), resulting in neuronal hyperactivity and subsequent shrinking of CA3 neuronal cell bodies (Yu et al., 1999; Xiao et al., 2001). The clearance of excess K⁺ in the extra-cellular space by astrocytes also leads to the swelling of glial processes (Walz, 1987), promoting the formation of peri-neuronal vacuoles in the CA3 region (Lassmann et al., 1984).

Compared with CA3 neuronal cell bodies, the change in SI ratios in CA3 neuronal dendrites (subregion 3) occurred later, and may be related to dendritic swelling secondary to the translocation of water molecules from the extra-cellular space into the cytoplasm via highly activated Na⁺/K⁺-ATPase (Schwob et al., 1980; Kempski, 2001).
The Na⁺/K⁺-ATPase is mainly located in CA3 neuronal dendrites (Pietrini et al., 1992), and its activity will be increased when KAR activation leads to excessive Na⁺ influx. It has been shown that Na⁺/K⁺-ATPase activity reaches its maximum 3 h after KA treatment, possibly driven by the elevated intra-cellular Na⁺ following KA-induced neuronal depolarization (Sztitra et al., 1987; Blanco and Mercer, 1998). Therefore, the KA-induced MR signal changes in the CA3 at the acute stage may reflect neuronal hyperactivity and ionic imbalance, with subsequent abnormal activation of intracellular enzymes and apoptotic pathways (Yu et al., 2001).

Both MRI and histology analysis indicated that the lesions in CA1 subregions developed later than those in CA3 subregions, suggesting possible different pathogenic mechanisms. In contrast to CA3 subregions, the peak SI ratios in CA1 subregions occurred 72 h after KA treatment. A recent study demonstrated that CA1 pyramidal neuron lesions in KA-treated rats may be the result of ischemic rather than excitotoxic effects (Sloviter, 2005). The KA-induced edema in the hippocampus may compress drainage vessels (Sperk et al., 1983), resulting in anoxic-ischemic damage and CA1 pyramidal cell death 3–4 days after ischemic insult (Nitatori et al., 1995; Charriaut-Marlangue et al., 1996). Therefore, the spongy morphology in the CA1 region after KA injection may be due to vascular expansions. Interestingly, only CA1 neuronal cell bodies adjacent to the vascular pathology were affected, suggesting CA1 neuronal damage is secondary to vascular pathology (Sloviter, 2005). In the present study, the absence of increase in c-Fos positive neurons in CA1 at 1–2 h post KA also indicates that the delayed CA1 lesion may be independent of acute neuronal activation, and is related to the KA-induced disturbances of microcirculation followed by anoxic-ischemic insult (Sperk et al., 1983).

In the present study, MR SI in the CA1 area was higher than in CA3 area at 120 and 168 h after KA injection, and was shown to be due to proliferating and hypertrophic astrocytes in the hippocampal area. As neuronal loss with subsequent reactive astrogliosis is similar to that observed in hippocampal sclerosis in human, and may lead to TLE in humans (Tokuhara et al., 2005), the higher SI in the CA1 area may serve as an early sign of hippocampal sclerosis.

In contrast to previous MR studies, KA-induced brain damage in the amygdala and piriform cortex was not observed in the present study. It may be due to differences in animal preparation. In the present study, rats undergoing MR experiments were anesthetized, while the rats in previous reports were awake. Electroencephalography study in waking rats showed that KA causes acute epileptiform activity in CA3, CA1 and other limbic structures (Ben-Ari and Cossart, 2000), consistent with our c-Fos results in waking rats. However, previous studies had shown that isoflurane can inhibit the neuronal glutamate release induced by KA (Zhu and Baker, 1996), selectively decrease the response of AMPA receptors to KA, and attenuate the spread of neuronal activation (Dildy-Mayfield et al., 1996). Because the spread of neuronal activation is known to play a crucial role in induction of remote brain damage after focal intra-cerebral KA injections (Ben-Ari et al., 1980), the absence of other brain area involvement in the present study may arise from the inhibition of the activation spread by isoflurane. A recent study using radiofrequency current density imaging showed that KA-evoked depolarization in anesthetized rats was restricted to the hippocampus (Beravs et al., 1999), consistent with the present findings.

In the present study we also used MEMRI for the first time to clarify KA-induced neuronal activation in vivo. KA-induced MEMRI signal in CA3 can be attenuated by diltiazem, a Ca²⁺ channel antagonist, and was accompanied with reduction of DWI SI and focal neuronal swelling in histopathology. It suggests that MEMRI can reflect local neuronal activation, and is correlated well with the KA-induced hippocampal damage. MEMRI has been used to reveal neuronal activation in response to different stimuli (Aoki et al., 2004a; Wadghiri et al., 2004; Yu et al., 2005; Kuo et al., 2006; Lu et al., 2007), and has been shown to detect activity-induced mossy fiber sprouting 2 weeks after KA-induced seizures (Nairismagi et al., 2006). The present study found that MEMRI signals were first observed in the CA1 pyramidal cell layer and the alveus, consistent with previous findings (Aoki et al., 2004b; Kuo et al., 2006). The alveus is the output projection from hippocampal neurons, and borders the wall of the lateral ventricles (Paxinos, 2003). Mn²⁺ infusion rapidly causes Mn²⁺ accumulation in the ventricles, eventually leading to Mn²⁺ diffusion from the ventricles into nearby tissues including the alveus (Aoki et al., 2004b). Mn²⁺-enhanced CA1 pyramidal cells may be the result of axonal transport of Mn²⁺ from the alveus into the CA1 region (Pautler, 2004). In the present study, MEMRI signal was also detected in the CA3 pyramidal cell layer in KA-treated rats, and can be attenuated by VGCC blocker. VGCCs are supposed to be key regulators in neuronal excitability and may be important components in ictogenesis and epileptogenesis (Khosravani and Zamponi, 2006; Weiergräber et al., 2006). Although KA directly activates Ca²⁺-permeable KARs on CA3 pyramidal cells, leading to Ca²⁺ influx into neurons (Coyle, 1983; Yin et al., 1999; Weiss and Sensi, 2000), the attenuation of both MEMRI signal and DWI SI by diltiazem indicated that VGCC activation may be responsible for KA-induced CA3 lesions, and may play important roles in epileptogenesis (Shitak et al., 2007). The early MEMRI signal around CA3 pyramidal neurons may suggest KA-induced neuronal activation secondary to VGCC activation, consistent with the present findings in DWI and c-Fos staining.

In conclusion, the present study demonstrates for the first time that the subtle hippocampal lesions that develop post KA treatment can be observed longitudinally using high-resolution MRI. The MR signal changes correlate well
with histopathological changes and may reflect cellular mechanisms of excitotoxicity, and early signs of hippocampal sclerosis. Using MEMRI and DWI, we also demonstrate the role of VGCCs in early KA-induced hippocampal damage. Thus, high-resolution MR along with MEMRI may provide invaluable insights into the pathogenic mechanisms underlying KA-induced hippocampal lesions, and can be applied in the future to clarify the long-term effect of seizures on hippocampus.

Acknowledgements
The authors are grateful for technical assistance from the Functional and Micro-Magnetic Resonance Imaging Center supported by the National Research Program for Genomic Medicine, National Science Council, Taiwan, ROC (NSC94-3112-B-001-005-Y). The authors deeply appreciate Dr Bai-Chuang Shyu for the c-Fos staining and explanations.

References